Matritech

Product Information

NMP22®Test Kit †
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Catalog Number: D1100
96 Determinations

Caution: US Federal law restricts this device to sale and distribution by or on the order of a physician or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.

Intended Use

The Matritech NMP22 Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of the nuclear matrix protein NMP22 in stabilized voided urine. The Matritech NMP22 Test Kit is indicated as an aid 1) in the diagnosis of persons with symptoms or risk factors for transitional cell cancer (TCC) of the bladder (cut-off ≥7.5 U/mL) in conjunction with, and not in lieu of, current standard diagnostic procedures, and 2) in the management of patients with transitional cell carcinoma of the bladder, after surgical treatment to identify those patients with occult or rapidly recurring TCC (cut-off >10 U/mL).

Contraindications, Warnings and Precautions

CONTRAINDICATIONS

- There are no known contraindications for the Matritech NMP22 Test Kit.

PRECAUTIONS

- For *In vitro* diagnostic use.
- Specimens used in this kit must be collected following the Matritech NMP22 Urine Collection Kit instructions and stabilized with the NMP22 Stabilizer according to Collection Kit instructions for use.
- Do not mix components from different kit lots.
- Do not use components after the expiration date.
- Use only plastic containers to process or store urine samples, calibrators, sample diluent or controls. Do not use any glass containers other than containers in which calibrators and controls are supplied.
- Refer to local regulations for the disposal of medical waste when disposing of any remaining kit reagents or specimens.
- Store the Matritech NMP22 Urine Collection Kit at a controlled room temperature of 50 to 70°F (10 to 25°C).
- Do not use the Matritech NMP22 Urine Collection Kit beyond the expiration date.

WARNINGS

- Handle all specimens as if capable of transmitting infection.
- Do not eat, drink, smoke or pipette by mouth where kit reagents are being handled.
- Wear protective laboratory devices such as gloves and laboratory coats when handling specimens and kit reagents; Wash hands afterwards.
- The calibrators, controls and sample diluent contain human albumin. Each lot of human albumin is tested and found to be negative for antibody to HIV-1, HIV-2, HbsAg and HCV by FDA approved tests. Because no test method can offer complete assurance that these reagents do not contain HIV, Hepatitis or other infectious agents, reagents should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen. [see CDC-NIH manual; Biosafety in Microbiological and Biomedical Laboratories, 1984 page 12-16].
- OPD tablets and Sulfuric Acid Stop Solution are known skin and mucous membrane irritants.
- The stabilizer found in the Urine Collection Container of the Matritech NMP22 Urine Collection Kit and the calibrators, controls and sample diluent found in the NMP22 Test Kit contain trace amounts of Fungizone™ and gentamicin as antimicrobial agents, which may be toxic if ingested.

Summary and Explanation of the Test

Nuclear matrix proteins (NMPs) make up the internal structural framework of the nucleus[1,2] and are associated with such functions as DNA replication, RNA synthesis, and hormone binding[3,4,5]. Further work has indicated that NMPs are involved in regulation and coordination of gene expression[4,6,7]. Later work by Fey and Penman demonstrated that NMP expression varied with cell type of origin[8]. This observation was followed by work showing that soluble NMPs could be detected in the serum of cancer patients in higher concentrations than were found in normal sera[9]. Most recently, Partin and colleagues demonstrated that the pattern of expression of NMP differed in normal prostate tissue, benign prostatic hyperplasia, and prostate cancer. Other work has identified specific NMPs for osteosarcoma, colon and breast cancer.

The antibodies contained in this assay recognize the head domain of NuMA, nuclear mitotic apparatus protein. NuMA has been shown to be present in malignant tissues at levels more than ten times higher than in normal tissue[10]. The NuMA antigen moiety detected by the Matritech NMP22 Test Kit is referred to as NMP22. The assay detects both complexed (>1000 kD) and fragmented (~30 kD) forms of NuMA in stabilized voided urine.

In the urine of healthy individuals, NMP22 is present at low levels. Patients with TCC present in the bladder have been shown to release higher levels of NMP22 into the urine. The assay is designed to quantify NMP22 in stabilized voided urine.

Principle of Matritech NMP22 Test Kit

The Matritech NMP22 Test Kit is an easy-to-use enzyme immunoassay in a 96-well microplate format. The assay employs two monoclonal antibodies that are specific for the nuclear matrix protein NMP22. Calibrators, controls and stabilized patient urine samples are reacted with an antibody coated onto wells of the microplate. After washing, the captured NMP22 antigen is reacted with a second antibody labeled with digoxigenin (DIG). After a wash, the digoxigenin-labeled antibody is detected with an anti-digoxigenin antibody coupled to horseradish peroxidase (HRP-SAD) using o-phenylenediamine (OPD) substrate. The reaction is terminated by the addition of 2 molar sulfuric acid (2M H_2SO_4). The concentration of antigen in the urine is directly proportional to the intensity of color developed and the actual concentration is determined from a standard curve. The standard curve is determined by the concurrent testing of the NMP22 Calibrators which nominally range in concentration from 0 to approximately 120 U/mL. The actual calibrator values are printed on the calibrator vial labels.

Reagents

MATERIALS PROVIDED IN KIT

NMP22 Calibrators **5 x 2 mL (lyophilized)**
Contains NMP22 (except cal #1) in a synthetic human urine solution containing human albumin, bovine proteins and Fungizone and gentamicin as preservatives. Calibrators range in concentration from 0 to ~120 U/mL. The actual concentrations are printed on the vial label.

NMP22 Tri-Level Controls **3 x 2 mL (lyophilized)**
Contains NMP22 in a synthetic human urine solution containing human albumin, bovine proteins and Fungizone and gentamicin as preservatives. Control ranges are printed on the vial label.

NMP22 Coated Microplate Strip Well **1 x 96 wells**
Each plate consists of twelve 8-well strips coated with mouse monoclonal anti-NMP22.

Digoxigenin Anti-NMP22 Reagent **1 x 20 mL**
Contains digoxigenin-labeled mouse monoclonal anti-NMP22 with goat, mouse and bovine serum proteins and ProClin300 as a preservative.

HRP-SAD Reagent **1 x 20 mL**
Contains horseradish peroxidase labeled sheep anti-digoxigenin with goat, mouse and bovine serum proteins, ProClin300 as a preservative and other stabilizers.

NMP22 Sample Diluent **1 x 10 mL (lyophilized)**
Contains a lyophilized synthetic human urine solution with human albumin, bovine serum proteins and Fungizone and gentamicin as preservatives.

Color Development Buffer **1 x 40 mL**
Contains hydrogen peroxide in a citrate-phosphate buffer with ProClin300 as a preservative.

100X Wash Concentrate **1 x 30 mL**
Contains a 100X concentrate of a non-ionic detergent in phosphate buffered saline.

OPD Tablets **4 Tablets**
Individual foil-wrapped tablets containing 10 mg of o-phenylenediamine and excipients.

Instructions for Use

REAGENTS REQUIRED BUT NOT PROVIDED

- Matritech NMP22 Urine Collection Kit, catalogue number D2000
- 2 Molar (4 Normal) Sulfuric Acid Stop Solution; see Assay Preparation

MATERIALS NOT PROVIDED

- 200 µL precision pipette with disposable tips
- Precision pipette with disposable tips to deliver 2 mL
- microplate reader (490 nm)
- microplate washer/aspiration device capable of delivering at least 310 µL per well of solution with a 10 second soak between aspirations (optional)
- centrifuge
- plastic test tubes to prepare OPD solution
- plastic test tubes to prepare specimens (centrifuging, or diluting specimens)

Storage Instructions

- Store kit components at 2-8°C.
- Components as packaged are stable through the expiration date printed on the kit box label.
- Bring all reagents to room temperature (18-25°C) prior to use.
- Immediately after use, store all reagents at 2-8°C except the diluted wash solution which should be kept at room temperature.
- Reconstituted calibrators, controls and sample diluent are stable for 14 days at 2-8°C or for 30 days when stored at -20°C or lower in single-use aliquots. Do not allow aliquots to experience more than one freeze-thaw.
- Keep foil packaging for OPD tablets sealed until ready to use.

Specimen Collection

PATIENT PREPARATION

Persons should not be tested with the Matritech NMP22 Test if they meet any of the following criteria:

- Have had a total cystectomy
- Less than five (5) days after an invasive procedure, such as cystoscopy or catherization

SPECIMEN COLLECTION

A single void of urine should be collected and **STABILIZED IMMEDIATELY** by the patient or medical personnel, using the Matritech NMP22 Urine Collection Kit, catalogue number D2000. Collection should be done between midnight and noon (0:00 to 12:00 hours) if it is for post-surgical patient management. DO NOT USE OTHER METHODS FOR COLLECTING URINE SAMPLES. INVALID NMP22 MEASURMENTS WILL RESULT FROM IMPROPERLY COLLECTED SAMPLES OR SAMPLES THAT ARE NOT PROPERLY STABILIZED.

1. Stabilized urine collected with the Matritech NMP22 Urine Collection Kit should be blue/green in color.
2. Stabilized samples may remain at room temperature (18-25 °C) for up to 96 hours after collection before being processed (see SAMPLE PREPARATION for storage times after processing). After 96 hours they must be stored at 2-8°C. **If shipping temperatures are expected to exceed 35°C, samples should be processed and shipped FROZEN on dry ice.**
3. Sodium azide or other preservatives should not be added to the samples as incorrect results may be obtained when analyzed with the Matritech NMP22 Test Kit.

SAMPLE PREPARATION

1. Process the stabilized urine specimen as follows:
   a) Ensure that each specimen is at room temperature (18-25°C) prior to processing.
   b) Centrifuge the total contents of the specimen in plastic tubes at 500 to 1000 x G for 10 to 15 minutes at 10 to 25°C to remove precipitates.
   c) Decant the supernatant into a separate plastic container.
2. Analyze each sample supernatant using the Matritech NMP22 Test Kit procedure.
3. Samples that have been centrifuged and decanted [processed] may be stored at 2-8°C for up to 1 week prior to measurement. Alternatively, samples may be stored at -20°C or lower. -20°C sample storage should be limited to 8 weeks as antigen loss has been reported in some samples after 8 weeks. For storage longer than 8 weeks, -80°C is required. Samples should be thawed at room temperature. Thawing at elevated temperatures (greater than 25°C) may result in loss of antigen activity. Avoid more than three freeze/ thaws.
4. Stored samples should be centrifuged and decanted a second time to remove any additional precipitates before testing.

Procedural Notes

- It is important that the user be familiar with the procedure.
- A standard curve must be established with every run.
- Disposable pipette tips should be used to prevent cross-specimen or cross-reagent contamination.
- Avoid interference caused by detergents or other contaminants of labware by thoroughly rinsing prior to use.
- Adherence to protocol incubation times and temperatures is necessary to achieve valid results.
- Avoid microbial contamination of reagents when removing aliquots from the vials. Microbial contamination should be suspected if the reagent solutions become cloudy or emit strong odor.
- Do not expose OPD reagents to light, any oxidizing agents or metal during storage or incubation.
- Acid Stop Solution is 2 Molar [4 Normal] Sulfuric Acid. Any other concentration of acid will produce adverse results. This acid may be purchased commercially or prepared from concentrated acid. See ASSAY PREPARATION.
- Do not place samples of urine, controls or calibrators in glass containers other than the containers in which the reagents were supplied.
- Do not allow the microplate to become dry during any part of the assay procedure.

Quality Control

Good laboratory practices include the use of control specimens within each assay to ensure that all reagents and procedures are performed properly. The Matritech NMP22 Test Kit contains a set of tri-level controls, which can be used to verify assay performance. Suggested concentration ranges for each control level are printed on the vial labels. Because each laboratory may obtain slightly different results, it is suggested that each laboratory establish its own range for each level of urine control.

Procedure

ASSAY PREPARATION

- Allow kit components and processed stabilized urine samples to equilibrate to room temperature (18-25°C) for 15 to 20 minutes before use.
- Gently swirl all reagents to ensure mixing prior to use.
- Verify the samples have been prepared as outlined in SAMPLE PREPARATION.
- Prepare Wash Solution from the 100 X Wash Concentrate. Allow 100X Wash Concentrate to reach room temperature (18-25°C). If any precipitate is present, warm the concentrate until the precipitate dissolves. Dilute the 100X Wash Concentrate 1:100 with deionized water. NOTE: 10 mL of concentrate makes 1000mL of wash solution. The vial contains 30 mL.
- Prepare 2M (4 N) Sulfuric Acid Stop Solution by adding 11.1 mL of concentrated sulfuric acid (18 Molar = 36 Normal) to a final volume of 100 mL in deionized water. Store at room temperature.
- Reconstitute each Calibrator and Control with 2 mL deionized water. Reconstitute the Sample Diluent with 10 mL deionized water. Recap and let stand at room temperature (18-25°C) for approximately 10 minutes. Invert and swirl gently, do not vortex. Let stand an additional 10 minutes before use. Be sure the lyophilized material is completely dissolved before use.
- Coated Microplate Strip Well foil package should be opened by cutting close to the package edge with the zip-lock strip. Remove strip wells not required from the plate frame. Return all unused, coated strip wells to the foil package along with the desiccant packet. Carefully reseal the foil package with the zip-lock closure and store at 2-8°C.

ASSAY PROCEDURE

1. Wash NMP22 Coated Strip Well Plate 3 times with the Wash Solution using either an automatic microplate wash aspiration system or manually. For the automatic washing system, settings should allow each well to fill completely (minimum 310 µL) with a 10-second soak prior to aspiration. After the three cycles are completed the wells should be free of residual wash solution. If necessary, tap the plate on absorbent paper.
   Proceed immediately to step 2.
2. Pipet 200 µL/well of each calibrator, control and sample into assigned duplicate wells. Incubate 2 hours ± 5 minutes at 18-25°C.
3. Wash plate 3 times with Wash Solution as described in step 1.
4. Pipet 200 µL/well of Digoxigenin Anti-NMP22 Reagent (DIG~22) to all assay wells. Incubate 1 hour ± 2 minutes at 18-25°C.
5. Wash plate 3 times with Wash Solution as described in step 1.
6. Pipet 200 µL/well of HRP-SAD Reagent to all assay wells. Incubate 30 minutes ± 2 minutes at 18-25°C.
   NOTE: During the HRP-SAD Reagent incubation, prepare the OPD Solution by transferring the required amount of Color Development Buffer (10 mL for half plate, 20 mL for whole plate) into a plastic test tube. Add one OPD tablet/10 mL of Development Buffer. Cover tube with foil and store in darkened area until ready to use. *Prepare this solution no more than 30 minutes before use.* The tablet(s) should be completely dissolved before use. Avoid contact of metal objects with this solution.
7. Wash plate 3 times with Wash Solution as described in step 1.
8. Pipet 200 µL/well of OPD Solution (mix well before use) to each assay well. Incubate 30 minutes ± 2 minutes at 18-25°C. *Place developing plate in a darkened area during color development.*
9. Stop the color development reaction by adding 50µL/well of 2M(4N) H_2SO_4 Stop Solution. Tap the plate gently to mix the acid with OPD. Place plate in darkened area at 18-25°C.
10. Read the reaction within 10 - 30 minutes of the addition of the Acid Stop Solution using a plate reading spectrophotometer that has been set at 490nm and blanked to zero absorbance with deionized water. NOTE: To eliminate the possibility of reading errors, the microtiter well used to blank the instrument should only contain deionized water.

Results

COMPUTER ASSISTED METHOD

Computer assisted data reduction may be used to calculate results. The performance data in this insert were calculated using a point-to-point curve fit. Curve fitting based on a linear regression analysis has also been validated. It is recommended that each laboratory selects either a point-to-point or linear regression curve fit method. Curve fitting based upon other non-linear analysis methods such as log-logit or 4-parameter logistic fit is not recommended.

The same curve fit method should be used to calculate results from different assay runs to ensure consistent values are obtained.

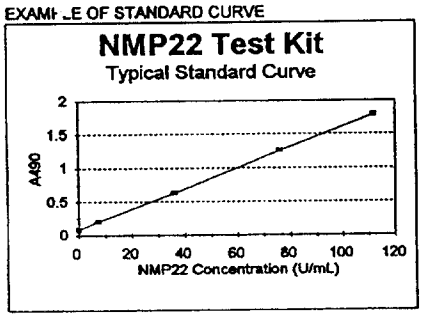
MANUAL METHOD

The standard curve may be constructed manually on rectilinear graph paper by plotting the mean A490 absorbance value for each set of calibrator replicates on the y-axis versus concentration in U/mL on the x-axis. The best curve fit (straight line) should be drawn through the data points. To determine the NMP22 concentration in a patient sample, find the point on the curve corresponding to the mean absorbance at 490nm of the patient sample and drop a vertical line to the x-axis. Read the concentration of NMP22 in units per milliliter (U/mL).

ELEVATED SAMPLE RESULTS

If the sample has a mean absorbance at 490nm greater than the mean absorbance of Calibrator #5, the sample should be diluted and re-run. Dilute the sample with the Sample Diluent so that the value obtained will fall within the range of the calibrator curve. When calculating the actual value of the diluted sample, the NMP22 value obtained in the assay should be multiplied by the dilution factor to obtain the actual NMP22 concentration. For example, if a sample is diluted 1 part sample to 1 part sample diluent (1 part sample in 2 parts total volume) the dilution factor is 2. If the diluted sample NMP22 value is 80U/mL, the reported value should be 160 U/mL (80 U/mL X 2).

EXAMPLE OF STANDARD CURVE



Calibrator		A490		
Units/mL	Rep 1	Rep 2	Mean	%CV
0	0.079	0.080	0.080	0.9
7.5	0.202	0.201	0.202	0.4
36	0.636	0.629	0.632	0.8
76	1.253	1.259	1.256	0.3
112	1.782	1.803	1.792	0.8

Samples	A490				Units/mL		
	Rep 1	Rep 2	Mean	%CV	Rep 1	Rep 2	Mean
Control I	0.200	0.190	0.195	3.6	7.7	7.1	7.4
Control II	0.441	0.433	0.437	1.3	23.4	22.8	23.1
Control III	0.797	0.783	0.790	3.1	46.4	44.2	45.3
Sample 1	0.122	0.122	0.122	0.0	2.7	2.7	2.7
Sample 2	0.417	0.421	0.419	0.7	21.8	22.1	21.9
Sample 3	1.326	1.382	1.355	2.8	80.8	84.3	82.6

ACCEPTANCE OF RESULTS

Calibrators:

The high calibrator (nominal 120 U/mL) should have an absorbance between 0.9 – 2.4.

The lowest calibrator (0 U/mL) should have an absorbance less than 0.15.

Tri-Level Controls:

1) Recovery of control concentrations should fall within established ranges.

REPORTING OF RESULTS

NMP22 values within the range of the standard curve may be reported to physicians. If NMP22 values are found to be greater than the high calibrator, it is recommended that the sample be diluted using NMP22 sample diluent and re-assayed. The re-assayed value, corrected for its dilution, should be reported.

Values of ≥7.5 U/mL were found to be optimal for identifying patients with a first occurrence of bladder cancer in the clinical trial reported within this insert. Values of >10 U/mL were found to be optimal for identifying patients at risk for occult or rapidly recurring bladder cancer in the clinical trial reported within this insert.

Expected Values

Management of TCC of the Bladder (cut-off >10.0 U/mL)

A prospective clinical trial was performed at 14 institutions and Matritech to determine the utility of NMP22 in identifying patients at risk for occult or rapidly recurring transitional cell carcinoma of the bladder (TCC) [11]. A total of 706 subjects were enrolled: 398 normal healthy volunteers, 117 subjects with benign urological diseases, 98 subjects with malignancies other than TCC, and 93 subjects with TCC who experienced at least 1 disease episode while enrolled in the trial. A disease episode is defined as the following: 1) performance of a surgical procedure for primary or recurring TCC, such as biopsy, fulguration, or transurethral resection of a tumor, or partial cystectomy or unilateral ureteronephrectomy; 2) collection of a urine sample according to the method described in the Matritech NMP22 Urine Collection Kit instructions, between 5 and 60 days after surgical procedure; and 3) performance of a procedure allowing assessment of presence of a neoplasm in bladder, urethra, ureters or pelvis of the kidney, such as cystoscopic examination or total cystectomy, between 2 and 6 months after the surgical procedure. A total of 128 disease episodes occurred among 93 subjects in the trial. To determine sensitivity and specificity, the disease episodes were classified as negative (no lesions seen on cystoscopy, or if a lesion was seen, pathologic examination of tissue indicated no abnormality present, or atypia or dysplasia), positive (pathologic examination of tissue indicated malignancy present), or unknown (lesion seen but no tissue collected for pathologic examination).

Samples were collected from normal healthy volunteers when each subject had no symptoms of a urologic abnormality and was not under a physician's care for a urologic condition, from subjects with benign urologic conditions when the subject was receiving treatment from a physician for that condition, and from subjects with cancers other than TCC when the subject was being treated or followed for that malignancy by a physician.

The percent distribution of NMP22 levels in self-referred healthy subjects, patients under treatment for benign diseases, and patients with malignancies of other sites is presented in the following table. The malignancy group includes those with active disease under treatment and those with malignancy diagnosed within the previous 18 months but which was no longer clinically evident.

	Percent Distribution of NMP22 (U/mL)					
	Number	0-10	>10-20	>20-50	>50-100	>100
Healthy Subject						
Male > 50 years	215	94.9%	3.3%	1.9%	0%	0%
Female > 50 years	151	87.3%	6.6%	4.6%	0.7%	0.7%
<50 years both sexes	32	90.6%	6.4%	0%	0%	0%
Total	398	91.7%	5.0%	2.8%	0.3%	0.3%
Benign Disease*						
UTI and Cystitis	26	84.6%	11.5%	3.8%	0%	0%
Urinary Calculi	16	93.8%	0%	0%	6.3%	0%
BPH & Prostatitis	52	92.3%	7.7%	0%	0%	0%
Other	37	83.8%	5.4%	8.1%	2.7%	0%
Total	117	88.0%	7.7%	3.4%	0.9%	0%
Cancers other than TCC/UT						
Head and Neck	6	83.3%	0%	16.7%	0%	0%
GI Tract	12	83.3%	0%	8.3%	0%	8.3%
Cardiovascular & Pulmonary	12	58.3%	8.3%	16.7%	16.7%	0%
Leukemia/Lymphoma	11	63.6%	18.2%	9.1%	9.1%	0%
Prostate	22	81.8%	0%	13.6%	4.5%	0%
Kidney (non-TCC)	18	77.8%	11.1%	11.1%	0%	0%
Other**	17	64.7%	17.6%	5.9%	0%	11.8%
Total	98	75.5%	10.2%	9.2%	4.1%	3.1%

Some patients are included in more that one category

***The "other" category in the cancers other than TCC/UT included malignancies of the bones, joints, cartilage, breast, uterine cervix, other female organs, testes and thyroid, pheochromocytoma, and hemangiopericytoma of the leg.*

In this study 92% of the healthy subjects had NMP22 concentrations of 10.0 U/mL or lower. Each laboratory should establish its own reference value.

Of the total 128 disease episodes (See EXPECTED VALUE for description of a disease episode) 116 could be classified as negative (no evidence of malignancy) or positive (occult or rapidly recurring malignant disease present). The first disease episode for each TCC subject in the trial was also analyzed; among 93 subjects, 87 had first disease episodes that could be classified as negative or positive. The following tables show the NMP22 results relative to a reference value of >10.0 U/mL for patients with occult or rapidly recurring TCC, following surgical treatment for TCC, and for patients with no malignant disease present.

These tables and analyses are broken down into the following categories: (1) all episodes of disease (multiple events per patient), (2) first episode of disease (3) sexes combined, (4) sexes separately, due to differences in NMP22 values seen between the sexes.

Sexes Combined; All Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤ 10 U/mL	10	64	74
> 10 U/mL	24	18	42
Total	34	82	116
		95% confidence level	
Sensitivity	70.6 %	55.3 - 85.9	
Specificity	78.0%	69.0 - 87.0	
Accuracy	75.9%	68.1 - 83.7	
Positive predictive value	57.1%	42.1 - 72.1	
Negative predictive value	86.5%	78.7 - 94.3	

Sexes Combined; First Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	6	46	52
> 10 U/mL	19	16	35
Total	25	62	87
		95% confidence level	
Sensitivity	76.0%	59.3 - 92.7	
Specificity	74.2%	63.3 - 85.1	
Accuracy	74.7%	65.6 - 83.8	
Positive predictive value	54.3%	37.8 - 70.8	
Negative predictive value	88.5%	79.8 - 97.2	

Males; All Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	8	58	66
> 10 U/mL	19	11	30
Total	27	69	96
		95% confidence level	
Sensitivity	70.4%	53.2 - 87.6	
Specificity	84.1%	75.5 - 92.7	
Accuracy	80.2%	72.2 - 88.2	
Positive predictive value	63.3%	46.1 - 80.5	
Negative predictive value	87.9%	80.0 - 95.8	

Males; First Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	4	43	47
> 10 U/mL	16	11	27
Total	20	54	74
		95% confidence level	
Sensitivity	80.0%	62.5 – 97.5	
Specificity	79.6%	66.9 – 90.3	
Accuracy	79.7%	70.5 – 88.8	
Positive predictive value	59.3%	40.8 – 77.8	
Negative predictive value	91.5%	83.5 – 99.5	

Females; All Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	6	8
> 10 U/mL	5	7	12
Total	7	13	20
		95% confidence level	
Sensitivity	71.4%	37.9 – 100	
Specificity	46.2%	19.1 – 73.3	
Accuracy	55.0%	33.2 – 76.8	
Positive predictive value	41.7%	13.8 – 69.8	
Negative predictive value	75.0%	45.0 – 100	

Females; First Disease Episodes (cystoscopic exam 2 to 6 months after Surgical Treatment)			
NMP22 (U/mL)	Occult/Rapidly Recurring Malignant Disease Present	No Evidence of Malignant Disease	Total
≤10 U/mL	2	3	5
> 10 U/mL	3	5	8
Total	5	8	13
		95% confidence level	
Sensitivity	60.0%	17.1 – 100	
Specificity	37.5%	4.6 – 71.0	
Accuracy	46.2%	19.1 – 73.3	
Positive predictive value	37.5%	4.6 – 71.0	
Negative predictive value	60.0%	17.1 – 100	

As indicated by the above statistical analysis, urinary NMP22 values of greater than 10.0 U/mL for samples collected following a surgical procedure may indicate occult or rapidly recurring malignant disease of the urinary tract. Patients with NMP22 values equal to or below 10.0 U/mL are less likely to have malignant disease on follow-up two to six months later.

Sensitivity, Specificity, PPV and NPV for Management of patients by Range of Incidence Rates				
Incidence Rate	Sensitivity	Specificity	PPV	NPV
10%	76.0%	74.2%	24.7%	96.5%
20%	76.0%	74.2%	42.4%	92.5%
30%	76.0%	74.2%	65.8%	87.9%

Diagnosis of TCC of the Bladder (cut-off ≥7.5 U/mL)

A second prospective clinical trial was performed at 33 sites to determine the utility of NMP22 as an aid in diagnosing transitional cell carcinoma of the bladder (TCC). Voided urine samples were collected from a total of 1147 individuals: 769 patients with unresolved hematuria or other symptoms or risk factors for bladder cancer (e.g. dysuria, exposure to carcinogens, history of smoking), 329 self-referred, hematuria negative, normal healthy volunteers, and 49 patients with active cancers other than those of the urinary tract.

To determine sensitivity and specificity, patients with risk factors for bladder cancer were classified as positive or negative for TCC. Patients were considered negative for TCC if their evaluation included a negative voided cytology, cystoscopy and upper tract evaluation (such as IVP or ultrasound). A negative voided cytology was defined as one in which no malignant or dysplastic cells were identified. A result of suspicious cells required further evaluation until the diagnosing physician deemed that no further diagnostic procedures were necessary at that time. Negative cystoscopy and upper tract evaluations were defined as those in which no tumor was identified, or if identified, was pathologically confirmed as non-malignant. Patients were considered positive for TCC if they had a positive cytology and/or cystoscopy and/or upper tract diagnostic procedure. A positive cytology was defined as one in which malignant or dysplastic cells were present. A result of suspicious cells required further evaluation. A positive cystoscopy was defined as one in which a tumor was seen endoscopically, and for which there was pathologic confirmation of TCC of biopsied or resected tissue. A positive upper tract evaluation was defined as one in which a tumor, filling defect, or wall thickening was identified and there was pathologic confirmation of malignancy of biopsied or resected tissue. No patients were found positive for upper tract cancer.

A single voided urine sample was collected from each patient with symptoms or risk factors for bladder cancer during their standard diagnostic evaluation. Samples from normal healthy volunteers were collected when each subject had no symptoms of a urologic abnormality, and had no history of a urologic disease during the prior twelve months. Samples were tested for hematuria by dipstick to rule out undiagnosed disease. Only samples that were negative for blood were included in the analysis. Samples from patients with other cancers were collected when the patients had clinically or pathologically confirmed malignancy and were not undergoing chemo-, immuno- or radiation therapy at the time of collection. These patients must not have been diagnosed with a urinary tract disease within the prior twelve months.

The percent distribution of NMP22 levels in hematuria-negative healthy subjects, persons with risk factors for TCC newly diagnosed with benign disease (as yet untreated), and persons diagnosed with cancers other than the bladder and not yet receiving treatment, is presented in the following table.

Percent Distribution of NMP22 (U/mL)							
	N	0–7.5	7.5-10	>10-20	>20-50	>50-100	>100
Healthy Subjects							
Males > 50	111	89.3%	5.4%	4.5%	0.9%	0.0%	0%
Females > 50	218	85.8%	5.5%	6.0%	2.8%	0%	0%
Total	329	86.9%	5.5%	5.5%	2.1%	0.3%	0%
Benign Disease							
UTI/Cystitis	58	86.0%	12.1%	0.0%	5.2%	5.2%	0%
Urinary calculi	71	84.5%	8.5%	8.5%	8.5%	4.2%	4.2%
BPH/ prostatitis	164	81.7%	4.9%	7.3%	2.4%	1.8%	1.8%
Other Benign Conditions	250	78.0%	7.7%	7.3%	2.3%	1.5%	3.1%
Total Benign Disease*	448	77.2%	7.8%	7.4%	3.3%	1.3%	2.9%
Other Cancers							
GI Tract	11	100%	0%	0%	0%	0%	0%
Leukemia/lymphoma	5	80.0%	20.0%	0%	0%	0%	0%
Prostate	21	90.5%	4.8%	0%	4.8%	0%	0%
Renal	1	0%	0%	0%	100%	0%	0%
Ovarian/Cervical	11	90.9%	9.1%	0%	0%	0%	0%
Total Other Cancers	49	89.8%	6.1%	0%	4.1%	0%	0%

Some patients are included in more that one category

The following table shows the percent distribution of the NMP22 results for final diagnoses of the 769 patients who had symptoms or risk factors for TCC.

Percent Distribution of NMP22 (U/mL)								
		N	0–7.5	7.5-10	>10-20	>20-50	>50-100	>100
Risk Factor patients	No Urinary Tract Disease	285	79.2%	8.3%	9.1%	2.8%	0.4%	0.4%
	Benign Urinary Tract Disease	448	77.2%	7.8%	7.4%	3.3%	1.3%	2.9%
	TCC	56	37.5%	10.7%	12.5%	16.1%	7.1%	16.1%

The following table shows sensitivity and specificity of NMP22 for this study for TCC using a cut-off of ≥7.5 U/mL.

NMP22: Sensitivity, Specificity, PPV and NPV for Risk factor Patients (T0-T4)				
	Sensitivity (95% Exact CI)	Specificity (95% Exact CI)	PPV (95% Exact CI)	NPV (95% Exact CI)
NMP22* (cut-off ≥7.5 U/mL)	62.5% (35/56) (48.5-75.1%)	78.0% (558/713) (74.8-81.0%)	18.2% (35/192) (13.0-24.4%)	96.4% (558/577) (94.5-97.7%)

*Compared to result of all three tests (cystoscopy, voided cytology, imaging): Positive=positive on at least one of the three tests; Negative=negative on all three tests.

The following table shows the sensitivity and specificity of voided urine cytology for this study for TCC.

Voided Cytology: Sensitivity, Specificity, PPV and NPV for Risk factor Patients (T0-T4)				
	Sensitivity (95% Exact CI)	Specificity (95% Exact CI)	PPV (95% Exact CI)	NPV (95% Exact CI)
Voided Cytology*	32.8% (15/46) (19.5-48.0%)	100% (713/713) (99.5-100%)	100% (15/15) (78.2-100%)	86.8% (713/744) (84.1-97.2%)

*Compared to result of all three tests (cystoscopy, voided cytology, imaging): Positive=positive on at least one of the three tests; Negative=negative on all three tests. Not every patient positive for TCC had a cytology result, but every patient negative for TCC did have a cytology result.

The following tables show the NMP22 results (cut-off ≥7.5 U/mL) compared to cytology results and the combination of NMP22 and cytology for the different stages and grades of TCC.

Percent and Fraction of Positives by stage(T0*-T4) (95% confidence interval)			
	NMP22 Cut-off ≥7.5 U/mL	Voided Cytology	NMP22 & Cytology Combined*
T0*	60.0% (3/5) (14.7-94.7%)	0% (0/5) (-)	60.0% (3/5) (14.7-94.7%)
Ta	45.0% (9/20) (23.1-68.5%)	18.7% (3/16) (3.8-41.4%)	87.5% (11/16) (33.5-78.8%)
Tis	80.0% (4/5) (28.4-99.5%)	66.7% (2/3) (9.4-99.2%)	100% (4/4) (39.8-100%)
T1	63.8% (7/11) (30.8-89.1%)	50.0% (5/10) (18.7-81.3%)	72.7% (8/11) (39.0-94.0%)
T2, T3, T4	76.9% (10/13) (46.2-95.0%)	85.8% (6/9) (21.1-99.3%)	92.3% (12/13) (64.0-99.8%)
Tx	100% (2/2) (15.8-100%)	0% (0/1) (-)	100% (2/2) (15.8-100%)

* Positive= positive on either test: Negative= negative on both tests

Percent and Fraction of Positives by Grade (95% confidence interval)			
	NMP22 Cut-off ≥7.5 U/mL	Voided Cytology	NMP22 & Cytology Combined*
No malignancy	60.0% (3/5) (14.7-94.7%)	0% (0/5) (-)	60.0% (3/5) (14.7-94.7%)
Low	50.0% (8/16) (26.0-74.0%)	13.3% (2/15) (1.7-40.5%)	86.3% (9/16) (29.5-80.3%)
Medium	70.6% (12/17) (44.0-89.7%)	42.9% (6/14) (17.7-71.1%)	82.4% (14/17) (56.6-96.2%)
High	88.8% (11/16) (41.3-99.0%)	38.3% (7/12) (27.7-84.8%)	87.5% (14/16) (61.7-98.5%)

* Positive= positive on either test; Negative= negative on both tests

CONCLUSION
Urinary NMP22 values equal to or greater than 7.5 U/mL in patients with symptoms or risk factors for bladder cancer may indicate the presence of TCC of the bladder. Patients with NMP22 values below 7.5 U/mL are less likely to have TCC.

Sensitivity, Specificity, PPV and NPV for Diagnosis of patients by Range of Incidence Rates				
Incidence Rate	Sensitivity	Specificity	PPV	NPV
1%	82.5%	78.0%	2.8%	99.5%
7.0%	82.5%	78.0%	17.9%	96.5%
7.3% (actual rate)	82.5%	78.0%	18.2%	96.4%
15.0%	82.5%	78.0%	33.4%	92.2%

Urine NMP22 concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the urinary tract without corroboration from other diagnostic procedures. Other clinically accepted tests and procedures should be considered in the diagnosis of disease and good patient management.

Performance Characteristics

LIMIT OF DETECTION
The lowest concentration of NMP22 antigen that can be measured reliably with the Matritech NMP22 Test Kit is 2.1 U/mL. The minimal detectable level is defined as that NMP22 value which corresponds to the absorbance that is two standard deviations (2SD) above the mean absorbance of twenty replicate determinations of calibrator #1 (0 U/mL).

PRECISION
Following procedures outlined in the National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-A; Evaluation of Precision Performance of Clinical Chemistry Devices, within-run and total precision were evaluated for three urine controls and five patient specimens. The specimens were assayed in duplicate in each of two independent runs repeated daily over a 20-day period.

Specimen	Number	Mean (U/mL)	Within Run %CV	Total %CV
Urine control 1	80	7.0	4.3	7.9
Urine control 2	80	25.8	2.5	4.8
Urine control 3	80	51.4	2.2	3.7
Specimen A	80	6.3	8.0	12.4
Specimen B	80	16.5	3.2	6.7
Specimen C	80	31.1	2.5	5.8
Specimen D	80	63.0	2.3	5.4
Specimen E	80	96.3	2.3	5.7

RECOVERY
Known concentrations of NMP22 antigen from stabilized patient urine were added to stabilized urine containing low endogenous levels of NMP22. The samples were measured in duplicate. The mean of 2 assays is reported. Mean recoveries of NMP22 in stabilized urine ranged from 89% to 111% with an overall mean of 99%. An example of a typical recovery study is summarized below:

NMP22 Added (U/mL)	NMP22 Recovered (minus endogenous)	Percent Recovery
48.8	45.8	94
31.5	28.4	90
23.5	22.3	95
11.0	11.4	103
	Mean Recovery	96

LINEARITY OF DILUTION
Six (6) stabilized urine samples containing elevated NMP22 levels were serially diluted with the NMP22 Sample Diluent (NMP22 Urine Calibrator 1 (0 U/mL)) and assayed in triplicate. Linear regression analysis of the NMP22 concentrations versus dilution was performed. The slopes for the 6 samples ranged from 0.80 to 1.06 with a correlation coefficient of greater than 0.993, thus demonstrating that the samples will dilute linearly.

POTENTIALLY INTERFERING SUBSTANCES
Substances listed were evaluated and found to have no significant effects on the results of the Matritech NMP22 Test Kit at the following concentrations:

Substance	Concentration
Urine Analytes	
Protein (Human serum albumin)	100 mg/dL
Protein (Human IgG)	100 mg/dL
Hemoglobin	1.6 mg/dL
Red Blood Cells (#/dL)	1.8×10^{11}
Whole Blood (v/v%)	1.0 %
Glucose	20.0 mg/dL
Cytokeratins (TPA)(U/dL)	40 U/dL
Therapeutic Agents	
BCG (Tice)(CFU/mL)	5.0×10^6
Thiotepa	60.0 mg/dL
Drugs	
Digoxin	0.05 mg/dL
Acetaminophen	20 mg/dL
Sodium Ascorbate	20 mg/dL
Caffeine	20 mg/dL
Sodium Salicylate	20 mg/dL
Sodium Acetylsalicylate	20 mg/dL
Ampicillin	20 mg/dL
Tetracycline	20 mg/dL

Limitations

- NMP22 concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the bladder without corroboration from other diagnostic procedures and should only be used in conjunction with other diagnostic information in the management or diagnosis of patients with transitional cell carcinoma of the bladder.
- Patients with known malignancy of the bladder or transitional cell carcinoma in other parts of the urinary tract may have urinary NMP22 levels within the range of concentrations observed in individuals with no known malignancy.
- For increased ability to accurately detect the NMP22 antigen, clinical studies investigating the use of NMP22 in the management of post-surgical TCC patients have shown that the urine sample must be collected between the hours of midnight and noon (0:00 to 12:00 hours) [10]. The clinical trial investigating the use of NMP22 in the initial diagnosis of patients with TCC did not restrict the collection time. For details on urine collection refer to NMP22 Urine Collection Kit Instructions for Use.
- Elevated urinary NMP22 levels have been observed in individuals with no known malignancy of the urinary tract. Occasional elevations have been observed immediately after extreme exercise (e.g. running more than 10 miles) in apparently healthy individuals, in some benign conditions (e.g. interstitial cystitis, urinary tract infections), in patients with renal cancer and malignancy of any site undergoing systemic chemotherapy. Elevated values are always seen in patients who have undergone total cystectomy. Significance of these elevated results is unknown. Physicians should use some judgement in determining when samples are collected.
- Samples collected fewer than 5 days after an invasive procedure such as cystoscopy or catherization of the urethra may result in elevated values due to tissue damage.
- Samples collected while the patient is undergoing intravesical therapy may not accurately reflect the presence or absence of malignancy in the bladder. Interpretation of NMP22 results from these samples has not been adequately determined.
- Only urine that has been stabilized with the NMP22 Urine Stabilizer should be used in this assay. Unstabilized urine and other body fluids should not be used in this test.

Ordering Information and Technical Services

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